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Obesity Management

Recent advances in adaptive thermogenesis: potential implications for the treatment of obesity

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Summary

Large inter-individual differences in cold-induced (non-shivering) and diet-induced adaptive thermogenesis exist in animals and humans. These differences in energy expenditure can have a large impact on long-term energy balance and thus body weight (when other factors remain stable). Therefore, the level of adaptive thermogenesis might relate to the susceptibility to obesity; efforts to increase adaptive thermogenesis might be used to treat obesity. In small mammals, the main process involved is mitochondrial uncoupling in brown adipose tissue (BAT), which is regulated by the sympathetic nervous system. For a long time, it was assumed that mitochondrial uncoupling is not a major physiological contributor to adaptive thermogenesis in adult humans. However, several studies conducted in recent years suggest that mitochondrial uncoupling in BAT and skeletal muscle tissue in adult humans can be physiologically significant. Other mechanisms besides mitochondrial uncoupling that might be involved are futile calcium cycling, protein turnover and substrate cycling. In conjunction with recent advances on signal transduction studies, this knowledge makes manipulation of adaptive thermogenesis a more realistic option and thus a pharmacologically interesting target to treat obesity.

Keywords: Calcium cycling, mitochondrial uncoupling, protein turnover, substrate cycling.

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Background

The fast growing prevalence of overweight and obesity in our society progressively affects public health. Obesity raises the risk of developing high blood pressure, diabetes type II and arteriosclerosis, all of which are risk factors for cardiovascular diseases. The problem of obesity has given a strong impulse towards metabolic studies. Small differences in energy expenditure might have large long-term effects on body weight (1). One of the suggested metabolic factors involved in the development of obesity is adaptive thermogenesis. It is defined as the regulated production of heat in response to environmental temperature or diet. It protects the organism from cold exposure and regulates energy balance (EB) after changes in diet (2). Recently, it has been shown that the individual metabolic responses to both mild-cold exposure and overfeeding are related (3). Therefore, cold- and diet-induced adaptive thermogenesis is likely to share the same regulatory mechanism. Therefore, it is feasible to aim more research at metabolic reactions to cold exposure.

Adaptive thermogenesis in response to cold exposure can be divided in two types: shivering thermogenesis (ST) and non-shivering thermogenesis (NST). The underlying mechanisms of NST and diet-induced adaptive thermogenesis are not fully elucidated yet. In this review we focus on these processes.

First, we discuss the metabolic responses after cold exposure and overfeeding and its respective neuronal regulation.

Thereafter, we describe the most likely potential mechanisms for adaptive thermogenesis.

Cold exposure

Back in the 1950s, it was already shown in rodents that oxygen consumption increased two- to fourfold after cold exposure (4). During daily cold exposure, shivering gradually decreased towards zero intensity in 20 d, while no decrease in oxygen consumption was found (5,6). This indicates the existence of NST. A few years later, similar results were found in man. During winter, when subjects were acclimatized to lower temperatures, energy expenditure increased about 25% upon cold exposure. After 10 d of cold exposure, shivering faded away, but energy expenditure remained elevated to the same level (7). This increase in energy expenditure during cold exposure without shivering can be considered to be the first proof of NST in humans.

The observed smaller amount of NST upon cold exposure in adult humans (compared with rodents) might be caused by the larger surface to volume ratio. Therefore, relatively less heat loss occurs with comparable core temperatures of approximately 37°C. Human newborns are able to increase their energy expenditure more than twofold without shivering (8), an increase comparable to that in rodents.

Most studies on cold exposure in humans are carried out in severe cold, when shivering also occurs (9,10). In these circumstances it is hard to make a distinction between ST and NST, as ST is superimposed over NST. However, before shivering starts, NST can be observed. During pre-shivering (room temperature of 15°C), resting metabolic rate increased by 12% (range –6% to 28%) (11). Also, attenuation of NST using medications has been performed. Administering propranolol, a non-selective β-adrenergic receptor blocker decreased oxygen consumption after cold exposure (room temperature of 5°C) with 26%, whereas there were no differences in shivering intensity (12). As propranolol inhibits the NST response (see chapter regulation), the decrease in energy expenditure is comparable to the amount of NST in the non-blocked status. In conclusion, these studies showed evidence for the existence of NST in adult humans.

In 1980, the same phenomenon has been described in humans after mild-cold exposure (22 vs. 28°C), without shivering. A mean increase in energy expenditure of 7% (range 2% to 12%) was observed (13). Recently, some other studies of mild-cold exposure in human subjects have been performed. After mild-cold exposure of 60 h, the total daily energy expenditure (TDEE) increased with 0.8 MJ d^{-1}. As no shivering was registered, the full metabolic response could be explained by NST (14). In this study, the range in inter-individual variation of the increase in energy expenditure was large, 0.15–1.45 MJ d^{-1}. In a comparable setting in the same lab, short-term (3 h) exposure to 15°C showed a significant increase in energy expenditure of 0.86 MJ d^{-1} (winter) and 0.57 MJ d^{-1} (summer), while the absence of shivering was confirmed by electromyogram measurements (15). The significant increase in metabolic rate in winter compared with summer conditions showed a cold acclimatization effect in the subjects. Considerable inter-individual differences in the metabolic response existed (–0.23 to 2.15 MJ d^{-1}), which remained throughout the seasons. Subjects that hardly increased their energy expenditure during summer were also low responders during winter and vice versa.

The above underlines that the metabolic response to cold exposure is an individual trait. A diminished energy expenditure is associated with an increase in body mass, while other factors remain fixed. Thus, low responders to cold might have a higher risk to gain weight than the subjects that have the ability to increase their energy expenditure, given an equal eating pattern. Claessens-van Ooijen *et al.* (16) recently showed that short-time mild-cold exposure (60 min at 15°C) resulted in a smaller increase in energy expenditure in obese subjects compared with lean subjects (6.4% vs. 17.2%). Keith *et al.* (17) proposed a possible relation between the recent increase in the prevalence of obesity and the fact that people nowadays live in a thermoneutral zone more often and, therefore, do not need to expend extra energy to achieve thermal comfort.

The most well-known mechanism to protect an organism against cold exposure is shivering. It can elicit increases in oxygen consumption up to five times basal metabolic rate (BMR) (18). Upon activation of the primary motor centre for shivering of the posterior hypothalamus, muscle fibres are starting to contract involuntarily (19). As no work is performed, heat is produced. However, as muscle fatigue occurs after longer periods of shivering, this is not an acclimatization mechanism for cold exposure but a protective mechanism to protect the organism from acute cold exposure. Therefore, shivering is not covered further in this review.

Overfeeding and underfeeding

After overfeeding, the same amount of excess energy intake does not invoke the same body weight gain in all people (20–25) (Table 1). In a classical study, Bouchard *et al.* (20) showed that overfeeding induced a weight gain of 4.3–13.3 kg after an excess energy intake of 353 MJ in 100 d. This implies a threefold range in energy cost of weight gain of 27–82 MJ kg^{-1}.

An important aspect in these studies is the level of compliance, as is discussed extensively in the *British Journal of Nutrition* after publication of the paper by Lammert *et al.* (23). Most of the studies mentioned above (20,21,23–25) maximized compliance by supervision during meals. Vomiting could be the only way to surpass the supervision; it is

Table 1 Weight gain ranges in a selection of overfeeding studies

Reference	Total excess energy intake	Weight gain (kg)		Cost of weight gain (MJ kg^{-1})	
		Minimum	Maximum	Minimum	Maximum
Bouchard *et al.* (20)	353 MJ (100 d)	4.3	13.3	27	82
Ravussin *et al.* (21)	60% of EB (9 d)	2.2	3.8	12.6	27.6
Diaz *et al.* (22)	50% of EB (42 d)	5.0	10.5	20.4	35.2
Lammert *et al.* (23)	105 MJ (21 d)*	–0.71	3.48	30	>105
	105 MJ (21 d)†	–0.73	3.17	33	>105
Levine *et al.* (24)	235 MJ (56 d)	1.4	7.2	32.6	168
Joosen *et al.* (25)	50% of EB (14 d)	0.19	3.0	69	>207

*High fat feeding.
†High protein feeding.
EB, energy balance.

not likely that all non-gainers in these studies did mislead supervisors and vomited. Furthermore, an underestimation of baseline energy requirements could be involved. The studies measuring baseline energy expenditure (21,24,25) ensured adequate weight maintenance energy intake levels, while studies assessing baseline energy requirements with questionnaires might have underestimated both baseline and overfeeding energy requirements (20,22,23). As both categories of estimation for baseline energy expenditure give similar ranges of cost of weight gain, no effect of the estimation method on weight gain is expected.

As energy intake was standardized in the studies mentioned above, the differences in weight gain have to be caused by a difference in diet-induced thermogenesis (DIT), the increase in energy expenditure in response to food intake. DIT can be divided into two categories: obligatory and facultative thermogenesis. The obligatory part of DIT consists of all processes related to the digestion, absorption and processing of food. The facultative component enables 'wasting' of energy after a high caloric meal and prevents the storage of energy. The inter-individual differences in weight gain can be explained by the variability in potency of this facultative component. Stock's (26) reanalysis of several studies showed even larger inter-individual differences after diets unbalanced in protein content. The larger cost of weight gain in these unbalanced diets presumably protects for deficiencies in underrepresented essential proteins. When there are deficiencies for a certain protein, more energy is expended, in order to be able to consume more, thus also more proteins, without too much weight gain.

The link between energy expenditure and weight gain after overfeeding in humans has been shown by Levine *et al.* (24). In an out-patient study giving 4.2 MJ of excess energy per day for 8 weeks, the increase in TDEE (on average 2.28 MJ d^{-1}) correlated negatively to the gain in fat mass ($r = -0.77$). Recently, a mean increase of 0.76 MJ d^{-1} was shown after 3 d of 60% overfeeding in the confined space of a respiration chamber. The inter-individual differences were large, ranging from –0.11 to 1.61 MJ d^{-1} (3). These differences in energy expenditure may correspond to the large inter-individual differences in weight gain after long-term overfeeding.

Reduction of adaptive thermogenesis can also be interpreted as a defensive, body mass saving, mechanism after underfeeding, as reviewed by Major *et al.* (27). Although over 80% of the variation in energy expenditure is explained by fat free mass, in several underfeeding studies (28–30) energy expenditure decreased below the expected value. In this case, adaptive thermogenesis prevents subjects from losing weight.

Neuronal regulation

Animal studies revealed that cold exposure, detected peripherally, is integrated by the hypothalamus, which activates the efferent pathways of the sympathetic nervous system (SNS). The SNS innervates (among others) thermogenic targets as the brown adipose tissue (BAT) and skeletal muscle (2). Several studies have shown that rodents with a blocked SNS or lacking catecholamines cannot maintain body temperature during cold exposure (31,32). Also, administration of β-adrenergic receptor agonists caused an increase in energy expenditure (50–150% increase, dependent on cold acclimation), comparable to the reaction to cold (31). Furthermore, storage of calories during normal caloric intake is increased after blocking the SNS (33).

In humans, comparable results have been found. Infusion of noradrenaline and adrenaline caused similar metabolic reactions as mild-cold exposure (24%–36% increase in BMR) (34). The sympathetic control of adaptive thermogenesis is mediated by β_1- and β_2-adrenoceptors, while energy expenditure is not affected by α_1- and α_2-adrenoceptors (35). The role of β_3-adrenoceptors in energy expenditure regulation, except in BAT, is not clear yet (36). Propranolol administration (a non-selective

β-adrenergic blocker) after glucose infusion induced a decrease in glucose-induced energy expenditure from 2.3 to 1.7 MJ d^{-1} (37), which is comparable with the reaction to overfeeding.

Tissues of interest

Skeletal muscle is potentially one of the largest contributors to adaptive thermogenesis in humans. An adrenaline infusion, which caused an increase of 25% in whole body energy expenditure, stimulated the forearm muscle to consume 90% more oxygen. Extrapolated to the whole body, skeletal muscle would account for about 40% of adrenaline-induced thermogenesis (38). Controversially, noradrenaline infusion did not increase muscle blood flow and decreased the arteriovenous oxygen concentration difference over the muscle (39). However, the authors stated, in their discussion, that the muscle blood flow measuring technique they used had the tendency to underestimate blood flow, which might have affected their results greatly. Furthermore, local concentrations of noradrenaline might not be large enough to provoke the thermogenic effect.

Results from ingestion of ephedrine, which is a sympathomimetic compound acting both centrally and peripherally, are in line with the abovementioned adrenaline study. Ephedrine ingestion resulted in an average increase in leg oxygen consumption of 25%. This accounted for an extrapolated contribution of the skeletal muscle tissue in ephedrine-induced thermogenesis of 50% (40). Finally, it has been shown that carbohydrates induced an increased adrenaline concentration, resulting in increased muscle thermogenesis (41). In conclusion, skeletal muscle tissue can be considered to be responsible for a large part of adaptive thermogenesis.

The BAT is the main contributor to adaptive thermogenesis in small mammals. Its relevance in adult humans has long been questioned. Despite the studies in the eighties showing a lack of a significant contribution of BAT (40), nowadays increasing evidence is found for a significant role of BAT in adult humans (42). Until now, no studies have been carried out quantifying the contribution of BAT to total adaptive thermogenesis.

Other tissues, such as the liver, which is highly metabolic active, might also contribute to adaptive thermogenesis in humans (2), although its contribution has not been quantified yet.

To gain more insight in tissues responsible for the increase in energy expenditure in adaptive thermogenesis, it is necessary to perform more rigorous tests. Combinations of measuring arteriovenous differences of oxygen or stable isotopes across tissues (43), micro-dialysis trials measuring metabolite concentrations in interstitial fluids of the tissues (44) and nuclear magnetic resonance spectroscopy (NMR) studies measuring selected substances with ^{31}P, ^{1}H, or ^{13}C (e.g. glycogen) (45) will reveal more information (46). In combination with cold exposure tests or β-agonist administration, relative contributions of tissues for adaptive thermogenesis can be calculated.

Postulated mechanism behind adaptive thermogenesis

Most reactions in energy metabolism are tightly regulated. An amount of fuel gives stoichiometric amounts of NADH and $FADH_2$. Fixed amounts of protons are pumped out of the mitochondrial matrix per molecule of NADH and $FADH_2$ (10 and 6 respectively). ATP synthase needs three protons to convert ADP and Pi to ATP. Finally, fixed amounts of ATP are used for cellular work (47). However, efficiency of these processes is not 100%, and energy is dissipated under normal baseline conditions (i.e. heat production). To enable an increase in thermogenesis following cold exposure without shivering, the efficiency of these processes has to be changed. Eligible processes for this energy dissipation are mitochondrial uncoupling, futile calcium cycling, protein turnover and substrate cycling. All processes will be discussed below.

Mitochondrial uncoupling

The most frequently studied mechanism is mitochondrial uncoupling in BAT, as it accounts for a major portion of thermogenesis after cold exposure in rodents (48). This uncoupling process is executed by uncoupling protein (UCP)-1, a unique inner-membrane protein for BAT. UCP-1 causes a reflux of protons into the mitochondrial matrix, bypassing the ATP synthase. Instead of using the energy stored in the proton gradient to produce ATP, which is the energy intermediate in the organism, heat is dissipated because of this so called proton leakage (48–50). UCP-1 knockout mice indeed cannot maintain body temperature in cold (48,51).

Until recently, BAT was commonly thought to be scarcely present in adult humans, in spite of studies indicating BAT in adult, cold acclimatized humans (52,53). In the 1980s, Astrup *et al.* (40) performed an elegant study in which they first examined the presence of BAT in human necropsies. BAT was most abundant in the perirenal region (92% of specimens contained brown adipocytes); smaller amounts were found in the cervical area (40%) and the pericardial fat depot (20%). They estimated the total content of BAT to be about 700 g. After stimulating thermogenesis with ephedrine in man, the authors showed in the same publication that the perirenal BAT was not as active as the rat BAT. Their calculations revealed that the 700 g of BAT could only account for 14% of the total increase in energy expenditure. Nevertheless, only one BAT depot was investigated, assuming that all depots have the same activity.

Similar results have been found by Cunningham *et al.* (54) by measuring BAT activity in isolated mitochondria from the same BAT depot. Following these studies, the attention for BAT in adult humans has been decreased.

However, several recent studies (55–57) that were performed with ^{18}F-2-fluoro-2-deoxyglucose-positron-emission-tomography/computer-tomography (FDG-PET/CT) showed active BAT-like depots when human subjects were exposed to cold (42). As no FDG-PET/CT measurements have been made with concomitantly taken BAT biopsies, it has not been shown directly that the active tissue identified as BAT-like depot is real UCP-1 containing BAT. However, the CT images revealed that the active sites are made up of adipose tissue, and several separate studies have shown UCP-1 containing BAT cells at active sites (42,58–61). With a combination of cold exposure, indirect calorimetry and FDG-PET/CT, the importance of these BAT-like depots for adaptive thermogenesis can potentially be quantified *in vivo* on whole body level, rather than at discrete locations using necropsy studies.

Recently, it has been shown that PRDM16, a protein abundant in BAT, is necessary for the activity of this thermogenic tissue (62). Transgenic expression of this gene in white fat precursors stimulated formation of brown fat cells, with UCP-1 expression and an increased uncoupled respiration (62). PRDM16 inhibits the formation of white adipose tissue and promotes the formation of BAT by binding to C-terminal-binding protein-1 and -2 and PPAR-γ-coactivator-1α and -1β (63). Therefore, it is likely that BAT can be recruited (even in adult humans) and can be a quantitatively important factor for adaptive thermogenesis. Testing for the abundance of this protein in (white) adipose tissue in human subjects can improve the insight in the presence in BAT. Surprisingly, PRDM16 has been shown to control a switch between brown fat and skeletal muscle cells (64), indicating that BAT is more similar to skeletal muscle tissue than to white adipose tissue (65). Expression of PRDM16 in myoblasts induced differentiation into brown adipocytes. UCP-1 containing BAT has been shown interspersed between muscle bundles of mice (66). In humans, brown adipocyte progenitors and UCP-1 mRNA have been identified in skeletal muscle tissue (67).

In humans four homologues of UCP-1 have been found that are abundant in other tissues than BAT. UCP-2 and -3 are more than 50% identical to UCP-1 (68,69) and do possess proton transport activity (68,70–73). UCP-2 is abundant in several tissues: spleen, lung, stomach and white adipose tissue (74). Therefore, it might be playing a role in adaptive thermogenesis, although the effect is expected to be small (75). Also UCP-2 was not up-regulated during mild-cold exposure, its predominant role is probably protection from reactive oxygen species (76). UCP-3 is an interesting target for research as it is predominantely expressed in skeletal muscle tissue. UCP-3 protein content was positively correlated to energy expenditure in humans. However, after 60 h of mild-cold exposure no increase in UCP-3 protein content could be found. UCP-3 mRNA was even down-regulated which would consequently lower the UCP-3 protein content (77). Alternative roles suggested for UCP-3 are not the regulation of energy metabolism but the handling of fatty acids in the mitochondria to prevent lipid-induced oxidative mitochondrial damage (78,79) and the reduction of the proton gradient to prevent production of reactive oxygen species (80). UCP-4 and -5 (or BMCP-1) are brain-specific (81,82) and have putative roles in the prevention of neuronal damage (83). Although expression of UCP-4 and UCP-5 was increased after cold exposure (84), they are not expected to play a role in adaptive thermogenesis as they are not expressed in peripheral tissues thought to be quantitatively important for adaptive thermogenesis (skeletal muscle, adipose tissue and liver).

Although the working mechanisms of mitochondrial uncoupling in skeletal muscle tissue are not yet fully understood, this does not imply that it is not a factor influencing adaptive thermogenesis. In a high resolution respirometry study, performed with permeabilized human skeletal muscle biopsies, it has been shown that during mild-cold exposure, state 4 (ATP synthase blocking by oligomycin) respiration, i.e. mitochondrial uncoupling, correlated significantly to the increase in energy expenditure (85). Changes in mitochondrial uncoupling in human skeletal muscle tissue have been shown before after endurance training (86) and triiodothyronine administration (87).

In conclusion, both UCP-1 mediated uncoupling in BAT and non-UCP-1 mediated uncoupling in skeletal muscle are candidate working mechanisms for adaptive thermogenesis.

Futile calcium cycling

Some fish living in cold environments (e.g. marlin and tuna) have an organ functioning specifically to dissipate heat. This organ warms muscle, viscera, brain and eyes (88). This organ does not have any UCP, as in BAT (89). The heater organ in fish is a derivative of muscle tissue and contains an extensive sarcoplasmic reticulum (SR) and T-tubule network. It lacks the contractile elements of the muscle tissue. SR calcium release channels, controlled by Ryanodine receptors (Ryr), cause a flow of Ca^{2+} out of the SR, triggered by acetylcholine receptors. The balance in calcium concentrations has to be corrected by an ATP-driven calcium pumping mechanism (Serca-1, sarco/endoplasmic reticulum Ca^{2+}-ATPase) (89). As Serca-1 uses ATP, which is not used for performing work, energy is dissipated in this futile cycle. The same mechanism has been found in humans suffering of malignant hyperthermia. Ryr-1 of these patients is more sensitive to several anaesthetic agents, leading to an outflow of Ca^{2+} out of the

SR, resulting in an compensatory ATP-driven influx, which produces excessive heat (90). In obese mice, it has been demonstrated that calcium cycling can be triggered with a selective CB1 (cannabinoid receptor 1) antagonist, with as a result increased energy expenditure and, consequently, weight loss (91). Cold exposure in UCP-1 deficient mice showed an increase in Serca-2a expression, which enabled a calcium cycling induced rise in energy expenditure (92). In rats, underfeeding resulted in a decrease in calcium cycling (compared with an EB condition), implicating that this inhibition served as an energy saving mechanism (93). Although no human data are available, calcium cycling is one of the eligible mechanisms for adaptive thermogenesis in humans.

Protein turnover

Protein turnover is defined as degradation of proteins into amino acids and resynthesis of new proteins. It is responsible for a large part of the energy expenditure in an organism, 15–20% of BMR (94). Most tissues do exhibit protein turnover, specifically the skeletal muscle tissue (25% of total protein turnover), liver (24%), skin (18%) and small intestine (15%) (94). Although the skeletal muscle has a slower protein turnover than the small intestine, it is still the major contributor because of its large tissue mass. As skeletal muscle and liver possess the largest adaptive thermogenesis capacity in humans, protein turnover could be a contributor to this process. However, studies in rats (95,96) and calves (97) did not find any increase in protein turnover upon cold exposure. After short-time cold exposure, protein synthesis even decreased. It is postulated that this is a mechanism for the organism to decrease lean body mass and herewith, BMR, to save energy under these harsh conditions (95).

On the other hand, it has been shown in humans after carbohydrate overfeeding that protein turnover increased by 12% (98). As inter-individual differences were large (5–25% increase in protein turnover), part of the differences in adaptive thermogenesis might be explained by this mechanism. Therefore, protein turnover might be quantitatively important, although no data is available on energy expenditure level.

Substrate cycling with fatty acids

Substrate cycling with fatty acids has been observed in patients after severe burn injury (99). In these patients, energy expenditure increased largely because of fatty acid cycling, next to the increase in protein turnover. In triglyceride-fatty acid cycling, fatty acids are released during lipolysis and subsequently re-esterified rather than oxidized (100). For both reactions different enzymes and ATP are used in a futile way. Recently it was discovered in animals that substrate cycling between *de novo* lipogenesis and lipid oxidation is stimulated by leptin, causing an increase in energy expenditure (101,102). It has also been shown in humans that mild-cold exposure increased fatty acid cycling (103). Upon starvation, it has been shown in an animal model that fatty acid cycling decreased, regulated by SCD1 (104). Therefore, it is feasible that fatty acid cycling does contribute to adaptive thermogenesis in humans. Several other substrate cycles exist, like the glucose/glycogen cycle, although they are not covered in this review, they may also affect adaptive thermogenesis.

Conclusions

Several mechanisms have the potential to contribute to adaptive thermogenesis in man. First, mitochondrial uncoupling in brown adipose fat tissue could be important, as increasing evidence arises that brown fat cells are present in adult humans and can be activated by cold exposure. Second, it has been shown that mitochondrial uncoupling plays a metabolic significant role in skeletal muscle tissue. This can be due to mitochondrial uncoupling in muscle fibres, to the possible switch of muscle tissue into brown fat or to another mechanism not elucidated yet. Furthermore, fatty acid cycling is also a promising target for future research, as it has been shown that it is increased by cold exposure in humans. As an increase in calcium cycling after cold exposure has not been measured in humans yet, it is still elusive if it has any influence in humans. Protein turnover is not found to be altered during cold exposure, although it increases during overfeeding, with large inter-individual differences. Therefore, this process could be of importance in diet-induced adaptive thermogenesis.

Although these mechanisms are potential contributors to adaptive thermogenesis, more research is needed to quantify their influence. Arteriovenous concentration difference, micro-dialysis and NMR studies in combination with cold exposure tests could reveal more tissues of interest and their relative contribution. Mitochondrial uncoupling in BAT can be investigated further with cold exposure tests in combination with PET/CT and PRDM16 measurements. Human mild-cold exposure tests measuring energy expenditure in combination with pharmacologically blocking or stimulating processes like calcium cycling, fatty acid cycling and protein turnover will give more insight in the relative contribution of these processes for adaptive thermogenesis. Furthermore, long-term cold acclimation tests could reveal whether uncoupling capacity can be increased. When more knowledge has been achieved, these mechanisms can be used for new strategies to prevent obesity, as they are all capable of increasing energy expenditure and, therefore, controlling long-term EB and body weight.

Conflict of Interest Statement

No conflict of interest was declared.

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